JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 111, G03013, doi:10.1029/2005JG000121, 2006

Alkenone producers inferred from well-preserved 18S rDNA in Greenland lake sediments

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Received 2 November 2005; revised 5 April 2006; accepted 9 June 2006; published 19 September 2006.

[1] The 18S ribosomal DNA (rDNA) sequences of haptophyte algae were successfully amplified using the polymerase chain reaction (PCR) from water filtrate, surface sediments, and a late-Holocene sediment sample (~1000 years old) from a group of lakes in the Søndre Strømfjord region of west Greenland. The DNA of the algal primary producer is extremely well preserved in the laminated lake sediments which have been deposited in cold (1°–2°C), anoxic, and sulphidic bottom water. Phylogenetic analyses of the Greenland haptophyte rDNA sequences suggest that alkenones in the Greenland lake sediments are produced by haptophyte algae of the class Prymnesiophyceae. The 18S rDNA sequences from the Greenland samples cluster within a distinct phylotype, differing from both marine haptophytes and from those reported previously from Ace Lake, Antarctica. The similarity of haptophyte rDNA sequences among all samples in this study suggests a single alkenone-based temperature calibration may be applied to these lakes for at least the past 1000 years. These sedimentary archives hold great promise for high-resolution, alkenone-based paleotemperature reconstruction of southern west Greenland, a region sensitive to atmospheric-oceanic climate phenomena such as the North Atlantic Oscillation (NAO).

Citation: D'Andrea, W. J., M. Lage, J. B. H. Martiny, A. D. Laatsch, L. A. Amaral-Zettler, M. L. Sogin, and Y. Huang (2006), Alkenone producers inferred from well-preserved 18S rDNA in Greenland lake sediments, *J. Geophys. Res.*, *111*, G03013, doi:10.1029/2005JG000121.

1. Introduction

1.1. Biomarkers and rDNA

[2] Lipid biomarkers extracted from geological samples have been commonly used to study past environmental change due to their resistance to diagenesis and sensitivity to environmental parameters [e.g., *Brassell*, 1993; *Meyers*, 2003; *Huang et al.*, 2004]. However, most lipid biomarkers are characteristic of a general group of organisms (e.g., terrestrial plants, algae, bacteria, and in the case of long-chain alkenones, certain members of the algal division Haptophyta) rather than a single organism, limiting the effectiveness of lipids for inferring the identity of specific producer organisms and environmental history. Ribosomal DNA (rDNA) comprises genes coding for ribosomal RNA (rRNA), which is associated with ribosomal proteins. In contrast to lipid biomarkers, the gene coding for small-subunit rRNA (18S in eukaryotes, 16S in prokaryotes) has been effectively used for phylogenetic reconstruction, with taxonomic resolution to the species level [*Sogin et al.*, 1986; *Edvardsen et al.*, 2000]. When such genes survive in the geologic record they can serve as exceptional biomarkers by offering species-level identification of producer organisms and reconstruction of the community structure of the ancient ecosystem [*Banning et al.*, 2005; *Coolen et al.*, 2004]. The rapidly expanding catalogue of published nucleotide sequences of rRNA genes from specific organisms (e.g., GenBank and RDP-II [*Benson et al.*, 2004; *Cole et al.*, 2005]) facilitates efficient identification of rDNA sequences obtained from environmental samples.

[3] Unfortunately, DNA molecules are relatively unstable in geological systems, limiting the widespread use of rDNA as a biomarker. Consequently, preservation of meaningful DNA sequences in most geologic environments has typically not been expected beyond 10 kyr [*Pääbo and Wilson*, 1991]. However, recent studies indicate excellent preservation of DNA in sediments of Ace Lake, Antarctica up to 10,000 years in age [*Coolen et al.*, 2004], and in Siberian permafrost up to 400,000 years in age [*Willerslev et al.*, 2003]. These findings suggest that DNA may survive in certain depositional environments for a considerable length of time and, in such instances, can serve as an unrivaled biomarker for studying ancient biogeochemical systems.

1.2. Lacustrine Alkenones in West Greenland

[4] Previous work by *D'Andrea and Huang* [2005] has shown that sediment from oligosaline lakes (1–3 psu) of

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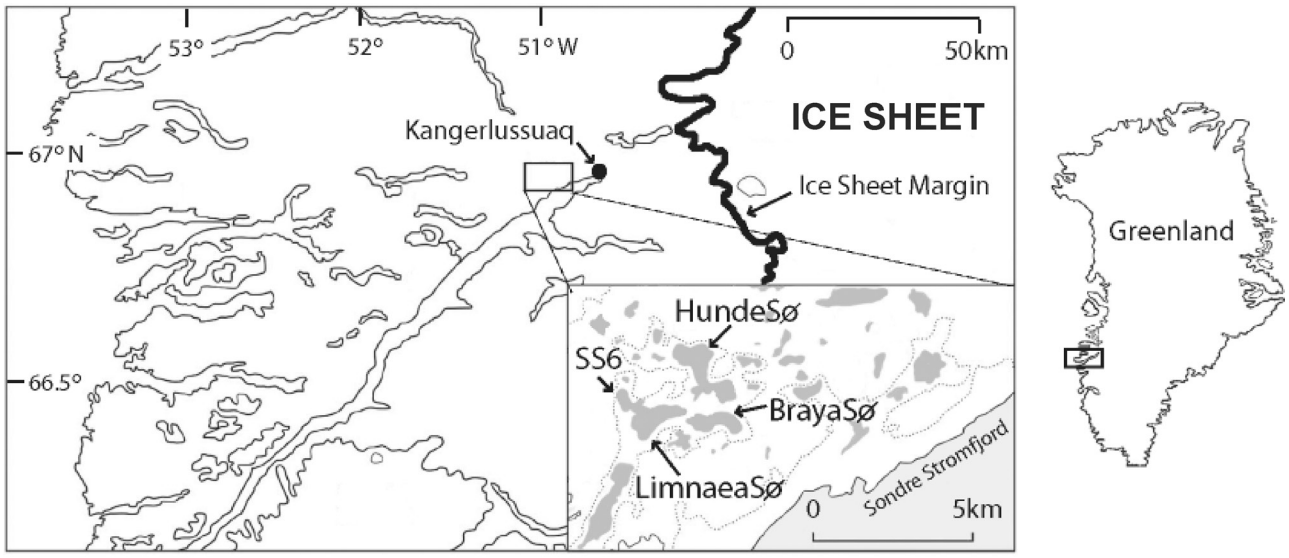

Figure 1. Site map showing study lakes in Kangerlussuaq, Greenland. Braya Sø, Hunde Sø, Limnæa Sø, and SS6 sediments all contain alkenones and 18S rDNA of haptophyte algae belonging to the class Prymnesiophyceae.

west Greenland contain exceptionally high abundances of long-chain alkenones. These important geochemical biomarkers are common in marine sediments and exhibit unsaturation patterns that are excellent sensors of temperature change [*Brassell et al.*, 1986; *Prahl and Wakeham*, 1987; *Brassell*, 1993]. Long-chain alkenones are specific to certain haptophyte algae (division Haptophyta, class Prymnesiophyceae [*Edvardsen et al.*, 2000]) and have been found in lake sediments from England [*Cranwell*, 1985], Antarctica [*Volkman et al.*, 1988], China [*Li et al.*, 1996; *Wang and Zheng*, 1998; *Sheng et al.*, 1999], Turkey [*Theil et al.*, 1997], Norway [*Innes et al.*, 1998], Germany, Russia, United States [*Zink et al.*, 2001], and South America [*Theissen et al.*, 2005]. Despite the ongoing discovery of lakes containing sedimentary alkenones, no one has been able to isolate and culture the lacustrine organisms responsible for producing these compounds.

[5] The objectives of the present study are to (1) assess the preservation of DNA in sediments of the west Greenland lakes, (2) use 18S rDNA to test for the presence of haptophyte algae in the Greenland lakes, (3) identify haptophytes in the lakes by rDNA comparison with known haptophytes, and (4) determine the similarity of haptophyte species among the study lakes by rDNA comparison. We conclude that alkenones in the west Greenland lakes are produced by haptophytes of the class Prymnesiophyceae, and represent a new phylogenetic clade. The similarity of 18S rDNA among the haptophytes in all lakes of this study supports the application of a single alkenone-based temperature calibration to the respective sedimentary records. The west Greenland lakes appear to preserve DNA of primary producers exceptionally well, probably due to the bottom-water conditions associated with meromixis.

2. Site Description

[6] The Søndre Strømfjord region (Figure 1) is situated between the western edge of the Greenland ice sheet and the Davis Strait and houses ~20,000 lakes. Most are dilute and oligotrophic, but there are several lakes with elevated salinity (1–3 psu) which are best described as oligosaline. Extensive work describing the water chemistry of the lakes of the Søndre Strømfjord region has been done by *Anderson et al.* [2001]. A summary of their findings concerning the major ion chemistry of the oligosaline and freshwater lakes is shown in Table 1a. The major difference in ionic composition between the oligosaline and freshwater lakes is the relative importance of calcium [*Anderson et al.*, 2001]. The amount of Ca with respect to other ions is greater in the freshwater lakes (Ca > Mg > Na > K) than in the oligosaline lakes (Na > Mg > K > Ca).

[7] The oligosaline lakes are ~110 m above the regional marine limit, ruling out seawater capture during isostatic uplift as a possible source of salinity. However, the major ion chemistry of the lakes implies that they have received ions through processes besides rainout. Nearby glacial outwash plains are a source of loess to the basins which likely delivers sodium to the lakes, while deflation of

Table 1a. Chemical Parameters of Søndre Strømfjord Lakes

	pH	Alkalinity (eq L^{-1})	Cl (eq L^{-1})	SO_4 (eq L^{-1})	Ca (eq L^{-1})	K (eq L^{-1})	Mg (eq L^{-1})	Na (eq L^{-1})
Oligosaline Mean[a]	8.76	11,302	10,827	3344	1697	3075	12,296	13,206
Freshwater Mean[a]	7.36	783	242	60	517	82	399	263

[a]Mean water chemistry data for oligosaline and freshwater lakes of the Søndre Strømfjord region from *Anderson et al.* [2001].

Table 1b. Physical and Chemical Parameters of Lakes From This Study[a]

Lake	Max Depth, m	Area, m^2	Salinity of Epilimion, ppt	Salinity of Hypolimnion, ppt	DNA Concentration,[b] μg/g TOC	Alkenone Concentration,[b,c] μg/g TOC
BrayaSø	23	686,100	0.9	1.9	780	82,700
HundeSø	35	1,836,600	1.5	2.3	790	50,300
LimnæaSø	18	1,118,600	1.4	2.8	1200	47,100
SS6	12	173,300	n.a.	n.a.	n.a.	11,100

[a]Here n.a. denotes not available. TOC is total organic carbon.
[b]Concentration in surface sediment.
[c]*D'Andrea and Huang* [2005].

uplifted marine deposits adjacent to Søndre Strømfjord represents a source of chloride [*Anderson et al.*, 2001].

[8] While all of the study lakes (Hunde Sø, Braya Sø, Limnæa Sø, and SS6; Figure 1) receive surface runoff during the spring melt, they are not connected by overland flow at any time of the year, with the exception of Limnæa Sø and SS6. These two basins have been physically connected to one another since 1998 [*McGowan et al.*, 2003] when rising lake water levels breeched a sill that had previously separated the lakes. All of the oligosaline lakes are closed basins, having no hydrologic outflow except for evaporation. Groundwater flow in the region is limited by the presence of permafrost.

[9] The enhanced salinity of the closed-basin lakes near the head of Søndre Strømfjord can be explained by the input of salts from local sources, and the negative effective precipitation of the region. The vicinity of the study lakes receives less than 150 mm of precipitation each year. Mean summer temperature is 9.3°C and mean winter temperature is −18°C (for the period 1961–1990 [*Box*, 2002]). *Anderson et al.* [1999] reported anoxic conditions in the hypolimnion of Braya Sø (Figure 1), owing to stratification of the water column.

[10] *D'Andrea and Huang* [2005] found exceptionally high concentrations of alkenones in sediments from four oligosaline lakes of the Søndre Strømfjord region (Braya Sø, Hunde Sø, Limnæa Sø and SS6; Figure 1 and Table 1b). The alkenone abundance suggests that the lakes provide a favorable habitat for certain alkenone-producing, haptophyte algae. However, the identity of such lacustrine haptophytes remains a mystery and despite numerous efforts, alkenone-producing haptophytes have not been observed from any alkenone-bearing lakes [*Li et al.*, 1996; *Theil et al.*, 1997; *Zink et al.*, 2001].

3. Methods

3.1. Field Work and Sampling

[11] Modern and late-Holocene sediment samples for DNA extraction were collected in August 2004 by Eckman dredge and gravity corer, respectively. The late-Holocene sample, collected from the deepest part of Braya Sø, is from a depth of 30 cm below the sediment-water interface and is estimated to be ~1000 years old by comparison with a ^{14}C-dated, neighboring gravity core [*Anderson and Leng*, 2004]. Sediment was extruded from the core barrel and a subsample for DNA extraction was removed from the center of the core to avoid potential contamination by younger sediments. Water filtrates were collected by filtration of 3 L of lake water using 0.22-μm Sterivex™ filters (Millipore). Water filtrates were immediately treated with prefiltered (0.22 μm) Puregene® lysis buffer (Gentra Systems, Inc.). Samples were kept cool and in the dark for ~12 hours before being frozen at −40°C. Sediment samples for alkenone analysis were collected by gravity corer.

3.2. Alkenone Extraction and Identification

[12] Details of alkenone extraction and analysis are given by *D'Andrea and Huang* [2005]. Briefly, samples were freeze-dried, homogenized, and extracted with dichloromethane (DCM). Compounds were analyzed by GC-FID for quantification, GC-MS for identification and GC-IRMS for isotopic analysis. Long-chain alkenones were identified by comparison of mass spectral data with previously reported standards and GC retention times [*de Leeuw et al.*, 1980; *Marlowe et al.*, 1984].

3.3. DNA Extraction and Amplification

[13] DNA was extracted from sediment samples using a FastDNA® SPIN Kit for Soil (Qbiogene, Inc.) according to the manufacturer specifications. Double-stranded DNA from extracts was quantified with a Beckman Coulter DU® 530 spectrophotometer by measuring the amount of light (at 260 nm, the wavelength of maximum absorption for DNA and RNA) absorbed by the extracted DNA. DNA was extracted from water filtrates using a Puregene Tissue Kit (Gentra Systems, Inc.). We used primers (Prym-429f: 5′-GCG CGT AAA TTG CCC GAA -3′, and Prym-887r: 5′-GGA ATA CGA GTG CCC CTG AC -3′) to amplify partial 18S rDNA from haptophytes [*Simon et al.*, 2000; *Coolen et al.*, 2004]. Polymerase chain reactions (PCR) were performed in a PTC-100™ thermocycler (MJ Research, Inc.). Reactions were performed as described by *Coolen et al.* [2004], and comprised initial denaturing for 4 min at 96°C, followed by 35 cycles including denaturing (30 s at 94°C), primer annealing (40 s at 55°C), primer extension (40 s at 72°C), and a final extension (10 min at 72°C). DNA extracted from two different isolated strains of the haptophyte *Prymnesium parvum* was used for positive control during PCR amplification and a blank sample was used for negative control. PCR amplicons were purified using a QIAquick® PCR Purification Kit (QIAGEN, Inc.) and cloned with a TOPO-TA cloning kit for sequencing (Invitrogen). Sequences of cloned rDNA amplicons were determined with an Applied Biosystems 3730xl DNA analyzer at the Marine Biological Laboratory. Ninety to ninety-five sequences of 426 nucleotides in length were obtained for each sample.

3.4. PCR Contamination

[14] Steps were taken to avoid and detect possible contamination of the late-Holocene sediment sample with contemporary DNA, or cross contamination among modern samples. All PCR reactions were conducted in a laboratory that does not routinely amplify 18S rDNA. Laboratory equipment was autoclaved or ethanol-washed prior to contact with a new sample and protective clothing was worn. All PCR steps, after DNA extraction, were conducted in a UV-sterilized bio-hood, and pipettes and pipette tips remained in this hood at all times. Additionally, all pipette tips were autoclaved prior to use. Negative controls were used throughout our experimentation to check for false positive results, and at no time yielded PCR products. Positive controls, in the form of haptophyte DNA from two strains of *Prymnesium parvum*, were also used throughout our experimentation. Because the positive control DNA is amplifiable with the primers we used, it served as a positive PCR amplification control while also providing a test for cross-contamination of samples. Sequences amplified from our positive control DNA could be easily distinguished from sequences amplified from extant DNA in our samples. DNA from the positive controls never appeared in any of the clone libraries, indicating the lack of cross contamination among samples.

3.5. Phylogenetic Analyses

[15] We used BLAST 2.2.10 [*McGinnis and Madden*, 2004] to retrieve 18S rRNA gene sequences from GenBank that were most similar to our environmental clone sequences and included them in our phylogenetic analyses. Sequences were aligned in ARB using the FastAligner option to the PT server database which had been built with the latest database available from the ARB website [*Ludwig et al.*, 2004]. The alignments were then manually adjusted. Sequences from the environmental samples were selected for the phylogenetic analyses by first defining operational taxonomic units (OTUs) at the 98% level with the program DOTUR [*Schloss and Handelsman*, 2005]. Representatives from each OTU were included in the analyses. The green algae *Pyramimonas olivaceae* and *Chlorella sp*. (GenBank accession numbers AB017122 and X72708) served as outgroups for the analyses. Our final data set consisted of 67 taxa and 380 unambiguously aligned positions.

[16] We used neighbor-joining analysis employing the Kimura 2-parameter algorithm [*Kimura*, 1980] as implemented in MEGA version 3.1 [*Kumar et al.*, 2004] to infer phylogenetic trees. The confidence of branching in the neighbor-joining analysis was assessed using 500 bootstrap resamplings of the data sets.

[17] We also subjected our dataset to a Bayesian analysis using MrBayes, Version 3.0b4 [*Ronquist and Huelsenbeck*, 2003], under the GTR model of substitution [*Lanave et al.*, 1984; *Rodriguez et al.*, 1990; *Tavare*, 1986] and considering invariants and a gamma-shaped distribution of the rates of substitution among sites. The chain length for our analysis was 5,000,000 generations with trees sampled every 100 generations using MCMC (Markov Chain Monte Carlo) analysis. Chain parameters appeared to be stationary after several thousand sampled trees; the first 10,000 trees (10^6 generations) were discarded as burn-in for the tree topology and posterior probability. We ran two replicate analyses that yielded comparable tree topologies and posterior probability values.

4. Results

[18] Haptophyte rDNA was successfully PCR-amplified from all sediment samples, including surface sediment from all four study lakes and a late-Holocene sediment sample (30-cm core depth, Braya Sø). Haptophyte rDNA was also amplified from water filtrate of one of the four lakes (Hunde Sø), but could not be amplified from water filtrates of the other lakes. Eight OTUs were identified among the Greenland sequences. Haptophyte rDNA in the west Greenland lakes does not belong to any haptophyte species previously catalogued in GenBank (Figure 2).

[19] The total amount of extractable DNA in surface sediment of the study lakes ranges from 600 to 1200 μg/gTOC (total organic carbon) (Table 1b), while the 1000-year old sediment sample (30 cm depth) contains $\sim$200 μg/gTOC of extractable DNA. These amounts are similar to the extractable DNA in sediment from Ace Lake, Antarctica [*Coolen et al.*, 2004].

5. Discussion

5.1. 18S rDNA Haptophyte Identification

[20] The successful PCR amplification of haptophyte rDNA indicates the presence of haptophyte algae in the west Greenland lakes. Phylogenetic analysis revealed the 18S rDNA-inferred relationship between the Greenland lake haptophytes and those catalogued in GenBank (Figure 2). The Greenland lake genotypes occupy a very strongly supported group (neighbor-joining bootstrap value of 92%; Bayesian posterior probability of 0.99) to the exclusion of all other haptophyte genotypes. Seven of the identified OTUs group within this "Greenland phylotype" and the OTUs do not indicate lake specificity. Approximately one percent of the environmental sequences (all from sediment of a single lake, Hunde Sø and representing the eighth OTU), did not occupy the Greenland phylotype, and instead grouped closely with *Isochrysis galbana* and certain haptophyte sequences from Ace Lake, Antarctica [*Coolen et al.*, 2004] in the neighbor-joining analysis (bootstrap value of 84%). All Greenland haptophyte 18S rDNA sequences group with haptophyte sequences from the algal class Prymnesiophyceae (Figure 2). The presence of prymnesiophyte rDNA in the lake sediment strongly implies that haptophyte algae are responsible for producing the alkenones found in Greenland lake sediments [*D'Andrea and Huang*, 2005]. It remains to be seen whether haptophytes of the Greenland phylotype, or the *I. galbana*-like haptophytes, or both, produce alkenones. However, given the large concentrations of alkenones in sediments of all of the lakes of this study and the fact that sequences belonging to the Greenland phylotype were found in all lakes (while the *I. galbana*-like sequences were only amplified from Hunde Sø sediment) it seems likely that members of the Greenland phylotype are alkenone-producers. The similarity of the *I. galbana*-like sequences to that of a known alkenone-producer suggests these haptophytes also produce alkenones. Further work will be needed to test these hypotheses.

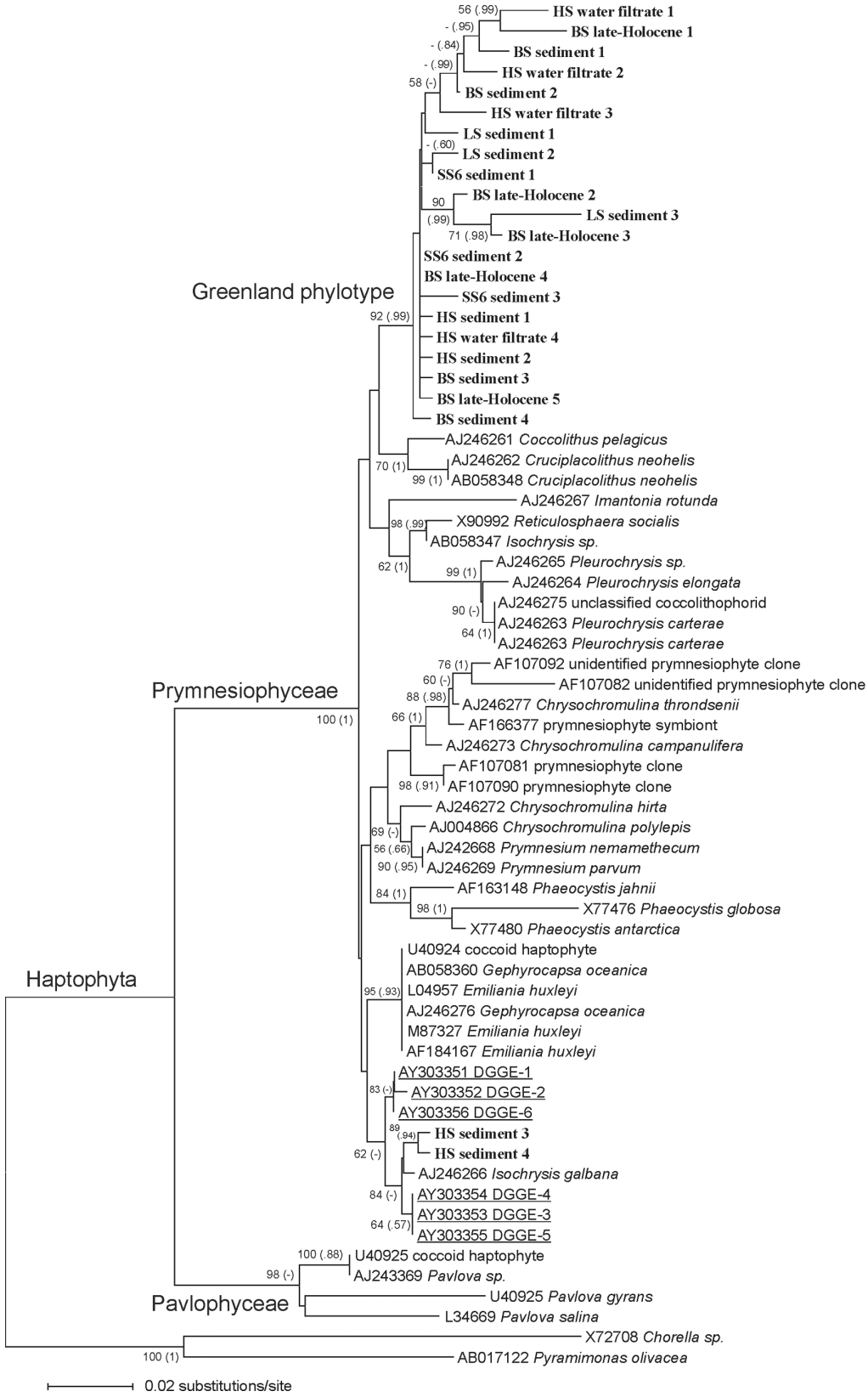

Figure 2. A consensus neighbor-joining phylogenetic tree depicting the 18S rDNA-inferred relationships between haptophytes in west Greenland lakes (boldface: HS, Hunde Sø; BS, Braya Sø; LS, Limnæa Sø), in Ace Lake, Antarctica (underlined: *Coolen et al.* [2004]) and haptophytes from GenBank (accession numbers are before genus names). Neighbor-joining bootstrap values greater than 50% are provided, followed by Bayesian posterior probabilities in parentheses. The evolutionary distance for the number of changes per site is represented by the scale bar.

5.2. Paleotemperature Implications

[21] Phylogenetic analysis revealed that haptophytes in all of the lakes are very similar (Figure 2). This was expected based on the similarity of alkenone distributions among the lakes (Figure 3) and the unusual presence of C_{38} methyl ketones in all study lakes [*D'Andrea and Huang*, 2005]. Genotypes from all four lakes (whether from surface sediment, late-Holocene sediment, or water filtrate) share a common phylotype, and show no finer-scale, lake-specific clustering that would suggest the presence of significantly different haptophytes among samples. The haptophytes appear to be the same within each lake, among the different lakes, and between ~1000 years ago and present. One exception to this general observation is found in the Hunde Sø surface sediment sample, where some of the rDNA sequences (5 out of 95) share a phylotype with *I. galbana* (as discussed above) rather than with the other Greenland sequences.

[22] The presence of two distinct haptophyte phylotypes in Hunde Sø, as determined by 18S rDNA sequences, may have important implications for understanding environmental changes and the habitat preferences of specific haptophytes. It is possible that under certain environmental conditions the *I. galbana*-like species could represent the dominant alkenone producer, while under other environmental conditions members of the Greenland phylotype become dominant. *Coolen et al.* [2004] proposed that phylotype shifts were an important mechanism for down core changes in alkenone unsaturation ratios in Ace Lake, Antarctica. Varying proportions of the *I. galbana*-like phylotype relative to the Greenland phylotype throughout the Holocene could represent a temperature-independent mechanism for changes in the alkenone unsaturation index. If this is the case, it may be necessary to apply different alkenone-based temperature calibrations to such intervals when reconstructing lake water temperature. Additionally, haptophyte community changes throughout the Holocene could reflect various aspects of environmental change (e.g., salinity). Further investigation into the evolution of haptophyte communities in the west Greenland lakes using quantitative PCR methods on ancient DNA could offer both biological and paleoenvironmental insights.

[23] Our inability to amplify haptophyte rDNA from water filtrates from three of the four lakes may indicate that the haptophyte algal bloom is not coincident with the sampling date. Ice melt and lake turnover occur between late May and mid-June [*Anderson and Brodersen*, 2001] but water filtrates were collected in mid-August. Perhaps the haptophyte bloom occurs in these lakes soon after ice melt, when nutrients are supplied to the photic zone by water column mixing. Hunde Sø, the lake from which haptophyte DNA was successfully amplified from water filtrate, has the longest fetch and the most wind mixing. This may result in more nutrient upwelling than occurs in the other lakes, extending the haptophyte growth period in Hunde Sø later into the summer. Future field work and sampling during the spring thaw will be needed to test these hypotheses.

[24] The similarity of haptophytes both spatially (among lakes) and temporally (between ~1000 years ago and present) in the west Greenland lakes suggests that a single alkenone-unsaturation temperature calibration can be applied to the different lake records. A culture-based, absolute temperature calibration has not yet been determined for lacustrine alkenones. However, using surface sediments from a series of German lakes, *Zink et al.* [2001] developed a lacustrine alkenone-based temperature calibration which, when applied to Greenland alkenones, provides good estimates of Greenland lake surface water temperature [*D'Andrea and Huang*, 2005]. The German lake calibration should therefore be applied to the Greenland lake records until development of a culture-based calibration.

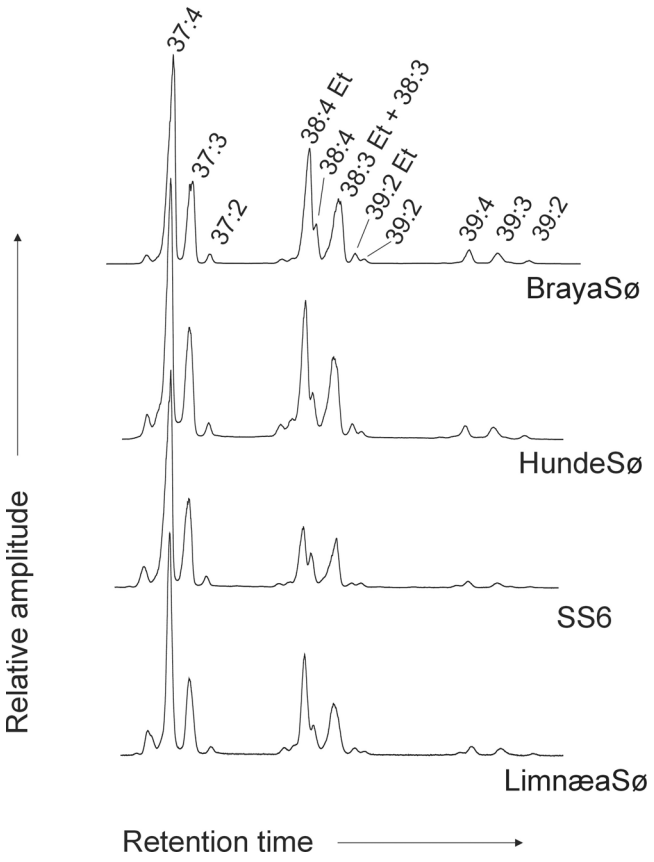

Figure 3. Gas chromatogram showing the similarity of alkenone distributions found in sediments of the four study lakes. The dominance of tetra-unsaturation is common for lacustrine alkenones, while the presence of C_{38} methyl ketones is unusual.

5.3. DNA Preservation in Lake Sediments

[25] Most ancient DNA studies have been carried out on fossil bone or tissue samples, and only a small number of studies have analyzed DNA extracted directly from sediments [*Coolen and Overmann*, 1998; *Willerslev et al.*, 2003; *Coolen et al.*, 2004]. The effectiveness of DNA as a biomarker hinges on the preservation of the molecule (more specifically, the fragment of interest) in the sedimentary deposit. Unfortunately, DNA is fairly labile and degradation via macromolecular chain breakage occurs readily in the natural environment. Hydrolytic and oxidative attacks on the phosphodiester bonds represent the most common degradation pathways for DNA [*Lindahl*, 1993; *Bada et al.*, 1999]. Furthermore, crosslink formations can cause PCR amplification blockage of environmental DNA sequences, and oxidative and hydrolytic modification of

nucleotide bases can lead to sequence errors [*Willerslev and Cooper*, 2005]. Kinetic calculations place the upper limit for preservation of meaningful DNA sequences in most geologic environments on the order of 10 kyrs [*Pääbo and Wilson*, 1991]. In cold environments this estimate increases to 100 kyrs, owing to slower reaction kinetics [*Poinar et al.*, 1996; *Smith et al.*, 2001], and under ideal conditions, 1 Myrs has been cited as the maximum duration for DNA preservation [*Willerslev and Cooper*, 2005].

[26] Certain qualities of a depositional environment may be more important than the age of the deposit with regard to the preservation potential of DNA. *Coolen et al.* [2004] found that 10 kyr old sediments from Ace Lake, Antarctica contain well-preserved DNA of algal primary producers that inhabited the lake's photic zone, and attributed the preservation to anoxic and sulfidic conditions at the bottom of Ace Lake. *Willerslev et al.* [2003, 2004] found DNA reflecting a diversity of taxa in 400-kyr-old permafrost and proposed that the exceptional preservation is due to the cold and anaerobic conditions associated with these deposits.

[27] The Greenland lakes have many qualities that favor the preservation of DNA. Anoxic conditions in the hypolimnia of the Greenland lakes should act to limit oxidative degradation of DNA. Dissolved oxygen approaches 0 mg/L at a depth of ~6 m in Braya Sø [*Anderson et al.*, 1999], which is 24 m deep. Thus particulate matter derived from primary producers in this lake settles through a largely anoxic water column, limiting the oxidative degradation of primary producer DNA in the water column as well as on the lake bottom. The presence of H_2S in the hypolimnia of the Greenland lakes should also limit oxidation by inducing the precipitation of metal ions (e.g., Cu^{2+} precipitated as copper sulphide) which otherwise facilitate DNA oxidation [*Eglinton and Logan*, 1991]. Perpetually cold temperatures at the bottom of the Greenland lakes (~1–2°C) act to slow the kinetic reactions responsible for DNA degradation [*Brodersen and Anderson*, 2000; *Willerslev et al.*, 2004]. The presence of clays and other clastic material in the lake sediments enables the adsorption of DNA to mineral surfaces, shielding DNA from enzymatic degradation, while elevated concentrations of monovalent (Na^+; ~26,000 eq L^{-1}) and, especially, bivalent cations (Mg^{2+}; ~21,000 eq L^{-1}) in the oligosaline Greenland lakes [*Anderson et al.*, 2001] likely facilitate the adsorption process [*Romanowski et al.*, 1991]. Along with excellent DNA preservation, the stratigraphic context provided by the Greenland lake sediment allows detailed, chronologic reconstruction of phytoplankton, as well as zooplankton, and fish communities, and provides the opportunity to closely examine relationships between evolution and environmental processes over the past several thousand years.

6. Conclusions

[28] The 18S rDNA analysis indicates that the large concentrations of alkenones previously identified in the study lakes of west Greenland are produced by haptophytes of the class Prymnesiophyceae. The haptophytes in these lakes occupy a new phylogenetic clade, distinct from those comprising the well-studied alkenone producers *Emiliania huxleyi*, *Gephyrocapsa oceanica*, and *Isochrysis galbana*. The overwhelming majority of west Greenland haptophyte 18S rDNA sequences (i.e., all but 5 of the ~540 sequences from this study) share a common phylotype, indicating the similarity of the alkenone producers in all four lakes studied. The presence of similar haptophytes implies that the same alkenone-based temperature calibration may be applied to all the lakes. The 18S rDNA sequences from a late-Holocene sediment sample share a phylotype with the sequences from modern samples, suggesting that the same temperature calibration is valid for at least the past 1000 years of the sediment record.

[29] DNA of the algal primary producer is well preserved in the sediment of the oligosaline lakes of the Søndre Strømfjord region. These lakes will provide a unique opportunity to investigate the evolution of haptophyte communities in concert with, and to the benefit of, alkenone paleothermometry. The 18S rDNA stratigraphy [cf. *Coolen et al.*, 2004] could reveal changes in haptophyte communities through time, elucidating the environmental preferences of different haptophytes and providing a test for the applicability of the modern alkenone-based temperature calibration for paleotemperature reconstruction. Additionally, the DNA of many other organisms should also be preserved in the sediments of the west Greenland lakes, recording the evolutionary history of other members of the lake ecosystem (e.g., other phytoplankton, zooplankton, macrophytes, fish), which will be of great value to the paleolimnologic reconstruction of these lakes.

[30] **Acknowledgments.** This work was supported by grants from the National Science Foundation (NSF0081478, 0318050, 0318123, 0402383, 0520718), NASA (NAG5-10665, NNG04GJ34G) and the American Chemical Society, Petroleum Research Fund (ACS-PRF38878-AC2) to Y. Huang. We thank Paul Zimba for providing *Prymnesium parvum* cultures and N. John Anderson for the use of field equipment. We also thank Annette Coleman and two anonymous reviewers for comments that helped improve the quality of this manuscript.

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